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ANTIANEMIC TREATMENT IN EX-
PERIMENTAL POLYCYTHEMIA

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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By
LOUISE HANSON MARSHALL

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ANTIANEMIC TREATMENT IN EXPERIMENTAL POLYCYTHEMIA¹

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The therapeutic value of liver in anemia is widely known. It has been suggested in a preliminary report (Hanson and De Savitsch, 1934) that the rôle of the liver may be more general than is indicated by its action in anemia, that the liver may function to reestablish the normal number of erythrocytes in the blood whether these be too low or too high. If the liver does act as a regulator, then its administration should in some degree be successful therapeutically in conditions of polycythemia.

To test this hypothesis, a sustained polycythemia was produced in experimental animals, which were then treated with liver. Red cell counts at frequent intervals revealed the correctness of the assumption.

Among the various methods of producing polycythemia experimentally may be mentioned the administration of germanium dioxide (Hammett and others, 1922), injection of serum from an animal in which the erythroblastic reaction is taking place (Carnot and Deflandre, 1906), and feeding a diet low in ash content (Swanson and Smith, 1932). The agent most frequently used is cobalt, administered as the chloride, nitrate or glutamate salt. Mice, guinea pigs and frogs have been found sensitive to cobalt (Sutter, 1934), while dogs, rabbits and rats have been used most extensively.

The hematopoietic effect of cobalt was first noticed by the Waltners in 1929. These workers found that injecting or feeding cobaltous salts produced an acute polycythemia in rats. The following year the abnormally red and hyperplastic bone marrow characteristic of the condition was seen in cobalt-fed dogs (Mascherpa, 1930). Orten and his co-workers (1932) administered 0.5 mgm. of cobalt per day to rats on a milk diet supplemented by salts of copper and iron. This treatment increased the red blood cell count, the hemoglobin and cell volume, while the leucocyte value was not significantly altered. Further work (Orten and others, 1933a) showed that manganese added to the régime not only appeared to stabilize

¹ A preliminary report of this work was presented before the American Physiological Society on March 30, 1934.

the polycythemia, but also alleviated any toxic effects of long-continued administration of cobalt. This latter finding has been confirmed by Kleinberg (1934) on rabbits. Later workers (Beard and Andes, 1934) claim that it is not necessary to add copper to the diet.

EXPERIMENTAL WORK: METHODS. The present experiments have been carried out over a period of twenty months. White rats from the stock colony were weaned at the age of 21 days and distributed among experimental and control groups as evenly as possible as to sex and litter. The groups were kept in separate cages bedded in sawdust. At the age of four to five weeks, the stock diet was discontinued, and whole milk supplied ad libitum, supplemented with cod liver oil and yeast. This diet permitted maintenance of health and a continued growth below that of normal rats. At the same time each animal received daily 2.5 cc. of a solution containing cobaltous chloride, cupric sulfate, ferric chloride and manganese sulfate in such quantities that each dose contained 0.5 mgm. cobalt, 0.5 mgm. iron, 0.025 mgm. copper, and 1.0 mgm. manganese. It was found convenient to

TABLE 1

Erythrocyte counts in millions per cubic millimeter with and without ether anesthesia

YOUNG NORMAL FEMALES	ANIMAL 1	ANIMAL 2	ANIMAL 3	AVERAGE
Without anesthesia.....	5.95	7.72	7.42	7.03
With ether one hour later.....	7.80	7.40	6.55	7.25
YOUNG NORMAL MALES	ANIMAL 4	ANIMAL 5	ANIMAL 6	AVERAGE
With ether.....	9.25	10.07	7.47	8.93
Without anesthesia one hour later.....	9.37	9.77	7.57	8.90

administer the solution by stomach tube. A control group fed the normal stock diet was run simultaneously with each series of experiments.

After two to three weeks on the milk-mineral régime, preliminary erythrocyte counts were made to ascertain the degree of polycythemia. These counts were done every other day until three of the same magnitude were obtained. The usual diluting pipet and improved Neubauer counting chamber were washed and dried between counts, so that the same ones served throughout the entire series of experiments. Hayem's solution was the diluting fluid, and three squares of sixteen on one scale plus two on the other were counted. By taking squares in the same position each time, choice was eliminated.

Blood was taken directly by heart puncture under light ether anesthesia. Comparative counts on the same animals with and without ether showed no appreciable difference in the numbers of circulating erythrocytes. These figures are presented in table 1.

Treatment was carried out over a period of two weeks, during which time the cobalt administration was continued and the erythrocytes were counted every other day. In a few cases, reticulocyte counts were made. These were vitally stained with brilliant cresyl blue, and the smears stained with Wright's stain.

RESULTS. The results of all experiments carried out as controls are presented in the first part of table 2, and are graphically represented in figure 1.

Normal control animals showed an erythrocyte count which steadily increased from an average of approximately 7.5 million cells per cubic

TABLE 2

Average experimental and control erythrocyte counts in millions per cubic millimeter

NUMBER OF RATS	CONDITION AND DAILY TREATMENT	CONTROL PERIOD			DAYS AFTER INITIAL TREATMENT						
		I	II	III	2	4	6	8	10	12	14
12	Normal—un-treated	7.98	7.81	7.82		8.55		8.12	8.29	8.82	8.42
8	Normal—liver extract	7.21	7.31	7.27		7.12		7.37		7.84	8.06
16	Polycythemic—saline	11.16	11.71	11.61	11.60	11.62	11.64	10.98	11.20	11.39	11.30
7	Polycythemic—kidney extract	10.27	10.86	10.93	10.60	11.29	10.80	11.03	10.56	11.24	10.43
4	Polycythemic—raw meat	10.88	11.15	10.88	11.24	11.41	10.96	11.08	11.20	10.51	11.42
23	Polycythemic—liver extract	10.90	11.44	11.86	10.98	10.64	9.93	10.67	11.39	11.85	12.01
12	Polycythemic—ventriculin	10.50	10.44	10.69	10.70	9.86	10.53*	9.81	10.18*	9.73	9.63
8	Polycythemic—raw liver	10.11	10.26	10.56	11.65	11.53	10.26	11.04*	10.14	10.24	9.80

* These figures represent experiments on reduced dosage.

millimeter to 8.5 million. This is a typical increase with age for rats (Donaldson, 1924). Intramuscular liver extract² injections in a group of eight normal animals produced no demonstrable effect upon the erythrocyte count, other than the normal increase with age mentioned above. This is contrary to the findings of Watkins (1928). Reticulocyte counts on two untreated normal animals were below 1 per cent.

Polycythemic control animals yielded preliminary values ranging from ten to thirteen million erythrocytes per cubic millimeter, with an average of

² The first experiments were performed with Lederle extract, which was unsatisfactory. We are greatly indebted to Eli Lilly and Company for a tested concentrated extract, which was used throughout the experiments reported at this time.

11.5 million, as compared to an average of 7.6 million for the normal controls. The counts increased with the length of time on the cobalt régime, as may be seen by comparing the three figures obtained for the preliminary control periods in all groups on table 2. Daily intramuscular injections of 0.25 cc. saline did not alter the count. The reticulocytes of two polycythemic control animals averaged 3.8 per cent.

Kidney extract³ was tested on a group of seven animals to determine whether the response found with liver extract might be obtained with that of another tissue. Daily intramuscular injections of 0.25 cc. kidney extract caused toxic symptoms and fluctuation in the erythrocyte count which at

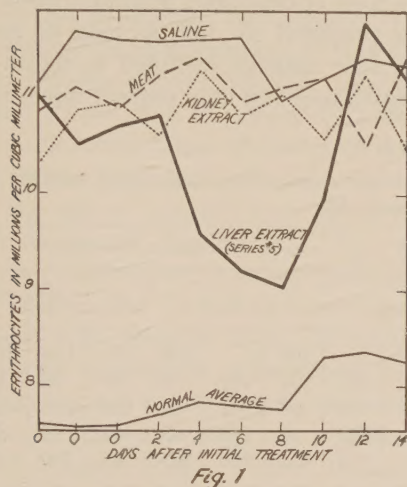


Fig. 1. Erythrocyte counts of normal and polycythemic control rats, from data of table 2. Included for comparison is the curve of a typical group of four polycythemic animals receiving liver extract.

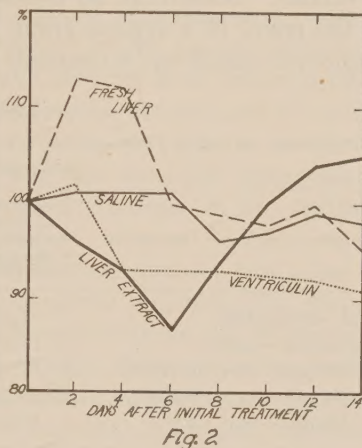


Fig. 2. Percentage variation in erythrocyte counts of treated and untreated polycythemic rats, taking the average of the control period counts as 100. Data from table 3.

no time fell below the initial polycythemic level for this group. By no means does this indicate that the anti-polycythemic action of the liver is specific, however, for the kidney extract was an alcoholic one, while that from the liver was aqueous.

The results with experimental groups are summarized in the second part of table 2. Table 3 and figure 2 present in percentages the variation from the control polycythemic level brought about by liver extract, ventriculin and fresh liver.

Liver extract was injected into the thigh muscles of a total of 23 poly-

³ Furnished by Abbott Laboratories, North Chicago, Illinois.

cythemic rats. The daily dose of 0.25 cc. represented the material from approximately 8.3 grams of fresh whole liver. This treatment caused a brisk but temporary fall in the red cell count, which in six to eight days amounted to from $1\frac{1}{2}$ to 2 million cells per cubic millimeter. At the lowest point, the average fall was to 87 per cent of the polycythemic control level. In a few cases the count fell to that of the normal controls. Among the animals tested, there were several found to be irresponsive to the treatment. This may be due to some absolutely refractory condition of the animals, or to a period of treatment too short to discover a slow response. The lowered erythrocyte count could not be maintained, the level rising again to an even higher value than before, in spite of continued administration of liver extract. Sometimes an increase preceded the fall, as may be seen from the curve of a typical group of four animals included in figure 1. Reticulocyte counts on two animals which received liver extract injections

TABLE 3

Percentage variation from control erythrocyte counts of normal and of treated polycythemic rats

CONDITION AND DAILY TREATMENT	CONTROL AVERAGE IN MILLIONS PER CMM. (= 100%)	DAYS AFTER INITIAL TREATMENT						
		2	4	6	8	10	12	14
Normal—untreated.....	7.57		103		102	109	110	109
Polycythemic—saline.....	11.49	101	101	101	96	97	99	98
Polycythemic—liver extract.....	11.40	96	93	87	94	100	104	105
Polycythemic—ventriculin.....	10.54	102	93	100*	93	97*	92	91
Polycythemic—raw liver.....	10.31	113	112	100	107*	98	100	95

* These figures represent experiments on reduced dosage, and for the sake of simplicity are not included on figure 2.

averaged 2.1 per cent. These counts were made when the treatment was having most effect, as shown by the erythrocyte level, and yield a value not quite half as great as that of the untreated polycythemic controls, but still higher than normal.

Since the response to liver extract was so spectacular, we were interested in seeing whether other antianemic agents would be successful. With this in view, 2.5 grams of hog gastric mucosa preparation⁴ were added to the milk-mineral régime of twelve polycythemic rats. The resulting fall in red blood cell values was not as great as that obtained with liver extract, but the lowered value was maintained throughout the experimental period. When the amount of ventriculin was decreased, as happened on the sixth and tenth days after the initial treatment (these figures are marked by asterisks on tables 2 and 3), the value rose again towards the polycythemic

⁴ Parke, Davis and Company's "ventriculin."

control level. Doubling the amount of ventriculin did not appreciably alter the response. Reticulocyte counts on two particularly responsive animals averaged 2.0 per cent, a value almost identical with that obtained for the animals receiving liver extract.

Raw liver was given to eight polycythemic rats, each receiving approximately 8 grams of fresh tissue per day. This amount corresponds roughly to the material administered per day in the liver extract injections. This resulted in an immediate further rise of 13 per cent in the already high erythrocyte count. By the sixth day the count returned to the control polycythemic level, around which it remained for the rest of the period. It might be possible that some of the potency of the fresh liver tissue was destroyed during digestion, so that the amount administered per os was not comparable to that injected. Reticulocyte counts on two animals averaged 1.4 per cent, a figure which indicates that raw liver was more effective than the other forms of treatment in suppressing the numbers of circulating immature red blood cells.

By way of control, four polycythemic rats were fed approximately 8 grams of raw, lean meat. This caused fluctuations in the erythrocyte count, but resulted in no significant change.

DISCUSSION. There can be no doubt that the increase in number of red blood cells brought about by cobalt is a true polycythemia, due to an increase in the rate of formation of new cells over the rate of destruction. That it is not a relative polycythemia, brought about by the concentration of erythrocytes through loss of plasma, is ruled out by the studies of Orten and others (1933b). These workers found that in chronic experimental polycythemia, there is an increase in the total blood volume, due to a rise in cell volume as well as to increased numbers of cells. The absence of large variations in the leucocyte count also eliminates the occurrence of a concentration of blood. The factor of mobilization of previously non-circulating cells has not been directly disproven, but the following considerations make it improbable. First, the macroscopically visible hyperplastic bone marrow, to which we have already referred, indicates increased activity on the part of the erythropoietic tissue. Secondly, the formation of new erythrocytes is indicated by our own increased reticulocyte counts of the untreated polycythemic control animals, and by the reticulocytes and dividing normoblasts found in the circulating blood of rabbits made polycythemic by cobalt (Klienbergh, 1934).

The mechanism by which cobalt produces a true polycythemia is unknown, but there are several clues at present. The most direct evidence is that of Berwald and others (1934), who have found that cobalt sulfate is unable to produce polycythemia in splenectomized rats. A few informal studies of our own indicate that the milk and mineral régime does not produce microscopically visible changes in the liver. The fact remains

that in some direct or indirect manner, cobalt stimulates the formation of erythrocytes in the red bone marrow.

It becomes interesting to compare cobalt polycythemia with the condition of primary polycythemia in humans (erythremia, Vaquez-Osler syndrome, splenomegalic polycythemia, polycythemia rubra). First described by Vaquez in 1892, and elaborated upon by Osler (1903), this usually fatal disease is characterized by an increase in blood volume accompanied by vascular engorgement of all organs. The increased blood volume is due to increased numbers as well as size of the erythrocytes (Deleopardi, 1934), while the leucocytes and other formed elements may or may not be increased. This blood picture relates the human disease to experimental polycythemia in animals. First descriptions of the disease are very positive about the fact that the red marrow is the only tissue showing hyperplastic changes. Careful searching by later workers (Delannoy, 1924; Letulle and Yacoel, 1924), has revealed evidences of hematopoietic activity in the spleen. Hirschfeld (1925) declares that even in light cases can hemopoiesis be seen in the spleen, in addition to the usual changes in the red bone marrow.

The condition of primary polycythemia has been ascribed to many causes (see Weber and Bode, 1929), but most writers agree upon the truth of Vaquez' original contention that the hematopoietic tissues are primarily at fault. Today, however, it is necessary to include the spleen as a hematopoietic tissue, in view of the autopsy evidence.

The suggestion of an hormonal control of the blood-forming tissues is not a new one. The early work of Carnot and Deflandre (1906) suggested this possibility. These investigators found that a small loss of blood conferred upon rabbits' sera an activity strongly hematopoietic when injected into new rabbits.

Hormones from several sources have been tentatively suggested as controlling hematopoiesis.⁵ On the basis of one clinical case, Morris (1933) presents "addisin," from the gastric mucosa, as a stimulator to the bone marrow. He found that his patient's polycythemia was made worse by intravenous injection of addisin, while lavage incidental to the treatment of duodenal ulcer brought a ten million erythrocyte count down to normal in six months. If addisin is comparable to or contained in ventriculin, then Morris' findings do not confirm our results with ventriculin in cobalt polycythemia. However, the difference in mode of administration may be a contributing factor.

Several writers have attributed the hormone to the liver. This hormone may play a stimulant or inhibitory rôle; if it stimulates the bone marrow, then polycythemia is held to be the result of hypersecretion; if it inhibits

⁵ For a discussion of the occurrence of polycythemia in endocrine disturbances, see Gunther (1929).

the marrow, then insufficient secretion would remove a check upon erythrocyte formation. The former view is that of Hirschfeld (1925), who supports it with the fact that anemic patients have been benefited by administration of serum possessing hematopoietic qualities after previous blood-letting. The latter view is supported by our own findings with liver extract injections, and may be invoked to explain the results of others.

Phillips (1933) saved a polycythemic patient, after other measures had failed, by irradiation of the liver. He attributes the cure to stimulation of the liver to take over the blood-destroying activity of the removed spleen. It is not impossible, however, that irradiation should have stimulated the secretion of a hormone which checked the overactivity of the red bone marrow.

Stephan (1930) found that both spleen and liver extract given orally produced a prompt response in the few polycythemic patients on whom it was tried. Stephan explains this not by assuming a hormone from the liver, which is stored in the spleen, but by assigning the origin of the material to the suprarenal cortex. He presents experiments with adrenalin-free cortin which brought about a slight drop in erythrocyte count in a few hours. However, the experiments seem too few in number and the change in red cell count too small to be conclusive.

Although we hesitate to add to the legions of dubious hormones already postulated for the liver, the balance of evidence indicates an hormonal control by the liver inhibiting the red bone marrow. The fact that we were not able to detect microscopic changes in the secretive elements of the liver, and that liver changes in the Vaquez-Osler syndrome have not been found, does not obviate the possibility that polycythemia is the result of a decrease in the amount of inhibitive liver hormone. On the other hand, it may be that the organism's need for the hormone has increased, and that there has been no compensation for the increased need, so that the normal supply becomes inadequate. The postulation that the liver and spleen act as a storage place for a hormone whose origin is elsewhere seems unnecessary.

Our results with ventriculin indicate that this substance contains the hormone or stimulates the liver to its production. The fact that the lowered erythrocyte count was maintained throughout the experiment by ventriculin leads us to believe that further experimentation with this substance would be more successful than that carried out along other lines. The temporary effect of liver extract, with a return to even higher figures, may indicate that we are dealing with two substances, of which the inhibitive one soon stimulates the development of an immunity to itself. Such a supposition makes possible an explanation of the absence of lowering effect of fresh whole liver per os, as the inhibitive substance may succumb to digestive processes. The hypothetical character of the explanations which we have assayed makes further research imperative.

We are especially grateful for the encouragement and assistance which Prof. Anton J. Carlson has given this work.

SUMMARY

A true polycythemia has been produced in rats by the administration of a milk diet supplemented by salts of cobalt, iron, copper and manganese. Red cell counts of blood obtained by heart puncture revealed a polycythemia of from 10.5 to 13 million cells per cubic millimeter, in contrast to 7.5 to 8 million for the normal controls.

The high erythrocyte count has been promptly lowered to an average of 87 per cent of its initial level within six days by daily injections of 0.25 cc. concentrated liver extract. Controls receiving injections of saline or kidney extract maintained a count averaging 11.5 million. The fall in erythrocytes was only temporary, a return to above the initial level occurring although the liver extract was continued.

Administration of ventriculin brought about a more gradual and less pronounced decrease in erythrocytes, which was maintained throughout the experiment.

Feeding of fresh whole liver caused a temporary increase in the already high erythrocyte count, but no lowering occurred. Fresh lean meat produced no change.

Certain similarities between cobalt polycythemia in rats and primary polycythemia as it occurs in humans have been discussed, and some of the theories of direct or indirect hormonal control of the erythroplastic tissues have been presented. The evidence presented is interpreted by assuming a hormone which originates in the liver and exercises an inhibiting action upon hematopoiesis.

REFERENCES

- BEARD, H. H. AND E. J. ANDES. *This Journal* **109**: 316, 1934.
BERWALD, W. P. E., J. H. ARSENEAU AND M. S. DOOLEY. *Proc. Soc. Exp. Biol. and Med.* **32**: 430, 1934.
CARNOT, P. AND C. DEFLANDRE. *Compt. rend.* **143**: 384, 432, 1906.
DELANNOY, E. *Arch. fran. Path. gen.* no. 10: 1, 1924.
DELEONARDI, S. *Cuore e Circolazione* **18**: 653, 1934.
DONALDSON, H. H. *The rat*. Philadelphia, 1924.
GUNTHER, H. *Endokrinol.* **4**: 96, 1929.
HAMMETT, F. S., J. E. MOWREY, JR. AND J. H. MUELLER. *J. Exp. Med.* **35**: 173, 1922.
HANSON, L. AND E. DE SAVITSCH. *This Journal* **109**: 48, 1934.
HIRSCHFELD, H. *Die Krankheiten des Blutes und der Blutbildenden Organe*. Vol. II, p. 259. Berlin, 1925.
KLEINBERG, W. *This Journal* **108**: 545, 1934.
LETULLE, M. AND J. YACOEL. *Arch. Mal. du Coeur* **17**: 65, 1924.
MASCHERPA, P. *Arch. ital. de Biol.* **82**: 112, 1930.
MORRIS, R. S. *J. A. M. A.* **101**: 200, 1933. *

- ORTEN, J. M., F. A. UNDERHILL, E. R. MUGRAGE AND R. C. LEWIS. *J. Biol. Chem.* **96**: 11, 1932.
 J. Biol. Chem. **99**: 465, 1933a.
 J. Biol. Chem. **99**: 457, 1933b.
- OSLER, W. *Trans. Assn. Am. Physicians* **18**: 299, 1903.
- PHILLIPS, H. B. *Med. J.* **133**: 393, 1933.
- STEPHAN, R. *Klin. Wehnschr.* **9**: 1068, 1930.
- SUTTER, J. *Comp. rend.* **116**: 994, 1934.
- SWANSON, P. D. AND A. H. SMITH. *J. Biol. Chem.* **98**: 479, 1932.
- Vaquez, H. *Bull. med.* **4**: 849, 1892.
- WALTNER, K. AND K. WALTNER. *Arch. exp. Path. u. Pharmacol.* **41**: 123, 1929.
- WATKINS, C. H., R. JOHNSON AND H. BERGLUND. *Proc. Soc. Exp. Biol. and Med.* **25**: 720, 1928.
- WEBER, F. P. AND O. B. BODE. *Polycythemia, erythrocytosis and erythremia.*
 London, 1929.

